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Sero-epizootological evaluation of Brucellosis in Bovines

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ABSTRACT

A total of 245 serum samples from 125 cattle and 120 buffalos were collected and screened for brucellosis. Screening by serological tests *viz.*, RBPT, STAT, EDTA- STAT, MET and dot-ELISA was done. The overall sero prevalence was recorded 19.00, 20.83; 20.00, 18.33; 14.40, 11.66; 17.60, 15.00 and 6.00, 0.00 per cent by RBPT, STAT, EDTA-STAT, MET and dot-ELISA in cattle and buffaloes respectively. Prevalence rate was found to vary with the test used. The predictive values obtained revealed no difference between the values of test and true prevalence in cattle, but little variation in buffaloes was recorded in terms of test employed. The findings of present investigation led to conclusion; that serological tests should be used in combination. The studies on point prevalence, test and true prevalence, mean titre values and predictive values of the serological tests shall be considered as markers in sero-epizootological evaluation of brucellosis in population.

Key words: Sero-prevalence, Brucellosis, Cattle, Buffalo, Epizootological markers.

INTRODUCTION

Brucellosis remains an important disease in animals and man throughout the world. In most part of the world the brucellosis eradication programmes have usually confined to bovine brucellosis. Cases of bovine brucellosis in organized herds of cattle, buffalo have frequently been reported in India (Kalorey *et al.*, 2000; Rathore *et al.*, 2002; Thakur and Thapliyal, 2002; Hussain *et al.*, 2003), but in rural area the status of brucellosis is not clear though there are scanty reports of sero-evidence of the disease. Serological tests are widely used in estimation of the status of disease, but none of the test gives accurate results. When these tests are used in combination along with the consideration of accurate sero-epizootological data, the limitations of each test could be minimized in case of sensitivity and specificity(Raju *et al* 2004). In view of these epizootological considerations, the present investigation was based on the systematic epizootological estimation of measuring disease occurrence and assessment of the predictive values of the serological tests for screening of brucellosis in cattle and buffalo in Nagpur region.

MATERIALS AND METHODS

A total of 245 serum samples were collected from 125 cattle and 120 buffalo from organized, unorganized farms and rural areas around Nagpur region and abattoir in Nagpur city. (Table 1). All the serum samples were subjected to evaluate sero- prevalence of brucellosis by applying standard tests viz., RBPT (Rose Bengal Plate Agglutination Test) (Alton, *et al.*, 1975), STAT (Standard Tube Agglutination

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Test) (Sharma, *et al.*, 1968), EDTA-STAT (Ethylene Diamine Tetra Acetic Acid Modified Standard Tube Agglutination Test) (Nielsen *et al.*, 1979), and MET (2-Mercaptoethanol Test) (Alton *et al.*, 1975). Of the samples tested by these conventional tests, 40 random samples each from cattle and buffalo were also subjected for dot-ELISA (DRDO, Gwalior). The titres were expressed in international units (I.U.). The animals with titre of 80 IU/ml and above were considered positive and those with antibody titre of 40 IU/ml were considered negative (Sharma *et al.*, 1968). The diagnostic RBPT antigen and *Brucella abortus* plain antigen strain 99 were obtained from IVRI, Izatnagar.

All the epizootological estimations were carried out as per the procedures described by Thursfield (1986). Measures of disease occurrence was estimated in terms of ;

a) Test and True Prevalence :

Some times events may recorded as being true, when actually they are not. This constitutes a false positive records and renders the diagnosis inaccurate. These errors and the validity of the diagnostic technique can be quantified by comparing results obtained by diagnostic methods with independent valid criteria using specificity and sensitivity of the test.

The sensitivity of a diagnostic method is the proportion of true positives that are detected by the method. The specificity of the method is the proportion of true negatives that are detected. Specificity and sensitivity can be quoted either as a probability between 0 and 1 or as a percentage. Specificity and sensitivity was calculated using following criteria;

Test Status	True Status		Total
	Diseased	Non-diseased	
Diseased	a	b	a + b
Non-diseased	c	d	c + d
Total	a + c	b + d	a + b + c + d
Sensitivity = a/(a + c)			
Specificity = d/(b + d)			

Prevalence in a population is always overestimated, and to measure this over estimated prevalence (P^T) *i.e.* prevalence by test in population, the following formula was used.

P^T = Prevalence x Sensitivity + (1- Prevalence) x (1- Specificity)

The corrected estimate of true prevalence (P) was then made by using the formula

$$P = \frac{P^T + \text{Sensitivity} - 1}{\text{Sensitivity} + \text{Specificity} - 1}$$

b) Mean titres-Logarithmic transformation of titres :

Pre-requisites for the calculation of Arithmetic Mean Titre (AMT) and Geometric Mean Titre (GMT) is to know the logarithmic transformation of titres. Serum is usually diluted in geometric series, that is with a constant ratio between successive dilutions. The commonest ratio is two. Thus serum is diluted 1/2, 1/4, 1/8, 1/16, 1/32 and so on. In the study, to avoid non-specific reactions due to high concentration of serum (prozone phenomenon), it was initially diluted by $\log_{10}$ (i.e.1/10) and then continuing in $\log_2$ dilutions (*i.e.*1/20, 1/40 ..... and so on). Values were divided by ten before taking logarithm to base two. The coded titres for the reciprocal dilutions

were then calculated.

Antibody titres expressed as reciprocal dilutions (X) and coded titres ($\log_2 X$) :

Reciprocal dilutions (X)	Code titre ($\log_2 X$)
1	0
2	1
4	2
8	3
16	4
32	5

To know the GMT from the coded titres the AMT was calculated using the formula.

$$AMT = \frac{\text{Sum of coded titres}}{\text{No. of titres}}$$

GMT is the antilog of the coded mean and was calculated using logarithm to base two ($\log_2$)

GMT = Antilog (AMT x 0.301)

c) Predictive Value of serological tests :

To assess the probability of *Brucella* positive animals by test is actually positive and test negative animals as true *Brucella* negative, the predictive value of the tests was calculated. In order to determine specificity and sensitivity of the test , the results obtained were analyzed using 2 x 2 contingency table. The predictive value was also assessed with the formula

$$\frac{P \times \text{Sensitivity}}{P \times \text{Sensitivity} + (1-P) \times (1-\text{Specificity})}$$

RESULTS AND DISCUSSION

The overall sero-prevalence of brucellosis reported in the present study was 19.20, 20.83; 20.0, 18.33; 17.6, 15.0; 14.4, 11.66 and 6.0, 0.0 respectively by RBPT, STAT, MET, EDTA-STAT and dot-ELISA in cattle and buffalo (Table1).

According to age, sex and area, prevalence rate was found to be varying with the test applied. Age wise prevalence was recorded in both cattle and buffalo (Table 2). Animals below one year of age were found more susceptible irrespective of the test employed except in MET. Suresh *et al,* (1993) reported animals aged above six years were found more susceptible to brucellosis, whereas, Thakur and Thapliyal (2002) reported animals between 0-3 years are more susceptible.

Observations on sex wise prevalence (Table 3) showed higher prevalence in female buffalos except in MET. In cattle, mixed findings were observed in different tests. Baby and Paily (1979), Suresh *et al.* (1993), Thakur and Thapliyal (2002) estimated significantly higher prevalence in females than males in cattle. Hussain *et al.* (1994) reported higher prevalence of brucellosis in female buffaloes.

In the present study, findings revealed that cattle from rural areas were found more susceptible to brucellosis by RBPT, STAT followed by cattle from organized farm by RBPT and MET, whereas reverse findings were observed by STAT and EDTA-STAT (Table 4). Prevalence rate ranged between 13-19%,

17-20%, 13-20% respectively in cattle from organized farms, unorganized farms, and rural areas by the test employed. Higher prevalence in organized farms and rural areas may be due to extensive practice of artificial insemination without adoption of hygienic and quality control measures. In buffaloes relatively less sero-prevalence was recorded from organized farms by all the tests, while mixed findings are noted in buffaloes from abattoir and rural area. Prevalence rate ranged between 5 – 16%, 9-25%, 13 – 22%, 11 – 20% respectively in buffaloes from organized farms, unorganized farms, abattoir and rural area by the test employed. Suresh *et al.* (1993), Thakur and Thapliyal (2002) also reported higher prevalence of brucellosis at organized farms in cattle. Prahlad Kumar and Singh (1997) observed 7.09 per cent sero-prevalence in buffaloes from abattoir. In the present study prevalence rate was found to be varying with the test employed and therefore these finding could not be well correlated with other reported studies.

The predictive value of the tests depends on specificity, sensitivity and prevalence. Sensitivity and specificity are innate characteristics of a test and do not vary, but the prevalence of disease in population being tested will affect the proportion of test positive animals that are actually diseased. The smaller the prevalence, larger the proportional over estimation and lower the predictive value of positive test result (Thursfield, 1986). To avoid the possibility of over estimation of prevalence by the test *i.e.* P^T the test prevalence was estimated using specificity and sensitivity, the innate character of the tests. The predictive values obtained (Table 5) revealed absolutely no difference between the true and test prevalence values in cattle by any of the test employed. However, in buffaloes the differences in the values were observed by RBPT, MET and EDTA-STAT. In the study no difference between P^T and P values are recorded by STAT. The results on assessment of P and P^T values indicate better prospect of STAT in terms of predictive values of positive results, since the test detect both IgM and IgG agglutinins and thereby always gives positive results (Sharma *et al.*, 1968).

The present study revealed considerable differences in GMT values both in cattle and buffalo by all the serological tests (Table 6a and 6b). Significance of GMT values in the present investigation is to know the possibility of recent infection and persistence of antibodies to estimate greater probability of infection in animals from different sources. The relative proportion of sero-positive animals, irrespective of titre and the GMT values of the sero-positive populations are considered while comparing coded antibody titres in population. High GMT value with similar prevalence rate in one farm than another indicates possibility of recent infection, whereas, similar GMT values with differences in prevalence rate indicates a much greater probability of infection at farm with high sero-prevalence rate (Thursfield, 1986). Comparison of GMT (Table 6a) values in cattle revealed more possibility of recent infection in organized farm A than B; and unorganized farm B than A. Amongst rural areas A, B and C, GMT values from animals of area A were suggestive of recent infection than that of B and C. GMT values also revealed possibility of recent infection from buffaloes (Table 6b) of unorganized farm A and rural area A, irrespective of the test employed. Such type of correlation studies of GMT's with stage of infection in cattle & buffalo species is not reported from the literature; however our earlier study on sheep and goat (Raju *et al.*2004) confirms significance of GMT values.

The specificity, sensitivity and diagnosability of the test were found to be varying irrespective of the species and are mentioned in Table 7 a and 7 b. The predictive values for sero-negative results in MET was always found to be more when compared with that of positive values irrespective of the species. The findings clearly indicate the utility of EDTA-STAT for assessment of true positive and true negative results. The present findings also raveled better prospects of dot-ELISA in screening of sero-negative and doubtful samples. The analysis of results in the present study proves the utility of all these tests in screening the animals for brucellosis in population as stated by Pralhad Kumar (1996), Hussain et al. (2003). The per cent predictive value (Table 8) for positive results ranged between 0.95, 0.80; 0.72,

0.63; 0.95, 0.66; 1.0, 1.0 by RBPT, STAT, MET and EDTA-STAT, respectively in cattle and buffalo.

The findings of the present investigation lead to the conclusion that, serological tests should be used in combination to estimate accurate sero-prevalence of brucellosis. The significance of EDTA-STAT for accuracy in confirming true positive and true negative results must be taken into consideration. Simultaneous studies on parameters viz., point prevalence, test and true prevalence, mean titre values and predictive values of the serological tests should be carried out to consider these parameters as markers in sero-epidemiological investigation of brucellosis in population.

Table 1. Per cent Prevalence of Brucellosis in Cattle and Buffalo.

Sr. No.	Species	No. of serum samples	RBPT	STAT	EDTA-STAT	MET	DOT-ELISA
1	Cattle	125	19.20	20.00	14.40	17.60	6.00
2	Buffalo	120	20.83	18.33	11.66	15.00	0.00

Table 2. Age wise Prevalence of brucellosis.

Species	Age group (Yrs.)	Prevalence rate (%)			
		RBPT	STAT	MET	EDTA-STAT
Cattle	Below 1 Yrs.	33.33	33.33	16.66	25.00
	1-3	28.00	28.00	24.00	20.00
	3-6	12.19	14.63	14.63	09.75
	Above 6	17.02	17.02	17.02	12.76
Buffalo	Below 1 Yrs.	28.50	14.28	28.57	14.20
	1-3	13.79	10.34	13.79	10.34
	3-6	22.85	25.71	17.14	14.28
	Above 6	22.44	18.36	12.24	10.20

Table 3. Sex wise Prevalence of brucellosis.

Species	Male/Female	No. of samples tested	Prevalence rate (%)			
			RBPT	STAT	MET	EDTA-STAT
Cattle	Male	33	15.15	21.21	15.15	21.21
	Female	92	20.65	19.56	18.47	11.95
Buffalo	Male	32	12.50	12.50	18.75	09.37
	Female	88	23.86	20.45	13.66	12.50

Table 4. Area wise prevalence of brucellosis

Species	Place	No. of samples	RBPT(%)	STAT(%)	MET(%)	EDTA-STAT (%)
Cattle	Org. farm	52	19.23	19.23	17.30	13.46
	Unorg.farm	29	17.24	20.69	17.24	17.24
	Abattoir	-	-	-	-	-
	Rural area	44	20.45	20.45	18.18	13.63
Buffalo	Org. farm	18	16.66	16.66	5.55	11.11
	Unorg.farm	32	25.0	18.75	12.5	9.37
	Abattoir	36	22.22	19.44	16.66	13.88
	Rural area	34	17.64	17.64	20.58	11.76

Table 5. Test and true prevalence in cattle and buffalo

Species	Test/True prevalence	RBPT	STAT	MET	EDTA-STAT
Cattle	P^T	19.2	20.2	17.6	14.4
	P	19.2	20.0	17.6	14.4
Buffalo	P^T	20.80	18.33	14.7	11.60
	P	20.83	18.33	14.7	11.60

P^T = Test prevalence
P = True prevalence

Table 6a. Arithmetic and Geometric Mean Titres in Cattle

	STAT			MET			EDTA-STAT		
Sources	Percent Prevalence	AMT	GMT	Percent Prevalence	AMT	GMT	Percent Prevalence	AMT	GMT
Org. farm									
A	11.53	3.83	14.22	11.53	3.50	11.31	7.69	3.00	8.00
B	7.69	2.20	4.59	5.76	2.00	4.00	5.76	1.50	2.82
Unorg.farm									
A	6.89	2.00	4.00	6.89	1.66	3.16	6.89	2.00	4.00
B	13.79	3.00	8.00	1.03	3.00	8.00	1.34	2.75	6.72
Rural Area									
A	6.81	2.75	6.72	4.54	4.50	22.62	6.81	3.00	8.00
B	6.81	2.66	6.32	6.81	2.33	5.03	2.27	1.66	3.16
C	4.54	2.33	5.03	6.81	2.33	5.03	4.54	2.00	4.00

Table 6b. Arithmetic and Geometric Mean Titres in Buffalo

Sources	STAT			MET			EDTA-STAT		
	Percent Prevalence	AMT	GMT	Percent Prevalence	AMT	GMT	Percent Prevalence	AMT	GMT
Org. farm									
A	5.76	2.33	5.02	5.55	2.00	4.00	11.11	2.50	5.65
Unorg.farm									
A	10.34	2.50	5.65	6.25	2.66	6.32	9.37	2.55	4.75
B	10.34	1.75	3.36	6.25	1.66	3.16	9.37	1.00	2.00
Rural Area									
A	5.88	2.50	5.65	8.80	2.50	5.65	2.94	3.50	11.31
B	11.76	3.00	8.00	11.76	2.50	5.65	8.22	2.50	5.65

Table 7a. Predictive Values of tests in Cattle and Buffalo

Test Status		RBPT			EDTA-STAT			MET		
		Positive Results	Negative Results	Total	Positive Results	Negative Results	Total	Positive Results	Negative Results	Total
Cattle	Positive Results	23 (a)	1 (b)	24 (a+b)	18 (a)	0 (b)	18 (a+b)	21 (a)	1 (b)	22 (a+b)
	Negative Results	2 (c)	99 (d)	101 (c+d)	7 (c)	100 (d)	107 (c+d)	4 (c)	99 (d)	103 (c+d)
	Total	25 (a+c)	100 (b+d)	125 (a+b+c+d)	25 (a+c)	100 (b+d)	125 (a+b+c+d)	25 (a+c)	100 (b+d)	125 (a+b+c+d)
Buffalo	Positive Results	20 (a)	5 (b)	25 (a+b)	14 (a)	0 (b)	14 (a+b)	12 (a)	6 (b)	18 (a+b)
	Negative Results	1 (c)	94 (d)	95 (c+d)	8 (c)	98 (d)	106 (c+d)	10 (c)	92 (d)	102 (c+d)
	Total	21 (a+c)	99 (b+d)	120 (a+b+c+d)	22 (a+c)	98 (b+d)	120 (a+b+c+d)	22 (a+c)	98 (b+d)	120 (a+b+c+d)

Table 7b. Predictive Values of tests in Cattle and Buffalo

	RBPT		EDTA-STAT		MET		STAT	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
Specificity d/(b+d)	0.99	0.95	1.00	1.00	0.94	0.99	0.93	0.92
Sensitivity a/(a+c)	0.92	0.95	0.72	0.63	0.54	0.84	1.00	1.00
Probability False positive b/(b+d)	0.01	0.05	0.00	0.00	0.06	0.01	0.007	0.07
False negative c/(a+c)	0.08	0.05	0.28	0.36	0.45	0.16	0.00	0.00
Predictive values Negative results d/(c+d)	0.98	0.99	0.93	0.1	0.90	0.96	1.00	1.00
Positive results (Diagnosability) a/(a+b)	0.95	0.80	0.92	1.00	0.95	0.66	0.72	0.63

Table 8. Comparison of Predictive value of tests

Species	RBPT		STAT		MET		EDTA-STAT	
	1	2	1	2	1	2	1	2
Cattle	0.95	0.95	0.72	0.78	0.95	0.94	1	1
Buffaloes	0.80	0.79	0.63	0.73	0.66	0.59	1	1

1 = Predictive value obtained using 2 x 2 contingency table.
2 = Predictive value obtained avoiding p^T (Prevalence overestimation) values.

REFERENCES

Alton, G. G., Jones, L. M. and Pitez, D. G. (1975). Laboratory Techniques in Brucellosis WHO, Monograph series **55.**

Baby, K. and Paily, E. (1979). Sero-epizootology of brucellosis in buffaloes in Kerala. Kerala J. Vet. Sci. **10** : 187 – 192.

Hussain, K. A., Saleem, A.N. and Fotooti, F. A. M. (1994). Prevalence of brucellosis in buffaloes, cattle and sheep in Mosul region. Iraq J. Vet. Sci. **7** : 238-239.

Hussain, S. A., Rahman, H., Pal, D. and Barman, N. N. (2003). Evaluation of serologic and delayed hypersensitivity tests for detection of bovine brucellosis. J.Vet. Publ. Hlth. **1** (2):165- 167.

Kalorey, D. R., Ingle, V. C. and Kurkure, N. V. (2000). Sero-prevalence of brucellosis in livestock and humans in Vidarbha region. Indian J. Anim. Sci. **70** (2) : 149 - 150.

Nielsen, K., Samagh, B. S., Speckmann, G. and Stemshorn, B. (1979). The bovine immune response to *Brucella abotus* II. Elimination of some sporadic serological reactions by chelating of divalent cations. Can. J. Comp. Med. **43** (10): 420-425.

Pralhad, Kumar. and Singh, D. K. (1997). Sero-prevalence of brucellosis in ruminants. 4th annual conference and National Symposium on Biotechnology in Animal Health and production for economic development in Asia respect to global scenario. 22nd -23rd Jan- 1997 pp-199.

Pralhad, Kumar. (1996). Evaluation of diagnostic test for serodiagnosis of brucellosis in animals and abattoir personnel. M.V.Sc. thesis submitted to IVRI, Bareilly (U.P).

Rathore, B. S., Barman, T. K., Singh, K. P., Singh, R. and Mehrotram, M. L. (2002). Microbiological and epidemiological studies on Brucellosis in organized herd and rural cattle and buffaloes of Uttar Pradesh. Ind. J. Comp. Microbiol. Immunol. Infect. Dis, **23** (2) : 195.

Raju,S., Kolhe, R..P., Raut, C., Shinde, S.V. and Zade, N.N. (2004). Seroepizootological studies on Brucellosis in Goat and sheep. J. Vet. Publ. Hlth . **2** (1-2): 1-6

Sharma, G. L., Sen, R. and Mathur, T. N. (1968). Diagnosis techniques in brucellosis *ICAR* Technical Bulletin, *A.H.* **9**. pp. 10.

Suresh, S., Ramkrishna, J., Saseedranath, M. R. and Bhan, M. N. (1993). Sero-survey of bovine brucellosis in Tamilnadu. Cheiron **22** : 1-7.

Thakur, S. D. and Thapliyal, D. C. (2002). Sero-prevalence of Brucellosis in various animal species. Vets. India (May-2002) 25-28.

Thursfield, M. (1986). Veterinary Epidemiology, Butterworth and Co. (Publishers) Ltd.